

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicants: Eijiro WATANABE et al.

Serial No.: 08/992,914

Group: 1638

Filed: December 18, 1997

Examiner: D.H.Kruse

For: RAFFINOSE SYNTHASE GENES AND THEIR USE

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Akitsu NAGASAWA, citizen of Japan and residing in Kamokogahara 3-28-56, Higashi-Nada-ku, Kobe-shi, Hyogo-ken, Japan, declare and say that:

1. I completed the master's course, with a major in agricultural biology, of the graduate school of Kyoto University and obtained a master's degree in agriculture at Kyoto University in March, 1984.

2. From April, 1984 to the present, I have been an employee of Sumitomo Chemical Company, Limited, the assignee of the above-identified application.

3. From April, 1984 to the present, I have been engaged in research works for plant engineering using recombination and other gene manipulation, such as cloning of plant genes, preparation and evaluation of transgenic plants.

4. I am one of the members of the research project related to the above-identified application and am familiar with the subject matter thereof.

5. I have read the Office Action mailed March 11, 2005 and the reference cited, and am familiar with the subject matter thereof.

6. To demonstrate successful identification of raffinose synthase genes in plant, I have made the following computer analysis.

ANALYSIS

1) The overall sequence homologies (%) among the amino acid sequences of raffinose synthases (RFSs), seed imbibition protein (SIP) and stachyose synthases (STSs) shown in Table 1 attached hereto were calculated based on a global multiple alignment (the alignment of sequences over their entire length) using the gene analysis software GENETYX-SV/RC for Windows version 6.1.0 (GENETYX Corporation; <http://www.sdc.co.jp/genetyx/>) with default parameters. The global multiple alignment was generated using CLUSTAL sequence analysis program. The amino acid sequences of the RFSs, SIP and STSs used to produce the global multiple alignment are as follows:

Sc-02:

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MAPPSITKTATLQDVISTIDIGNGNSPLFSITLDQSRDFLANGHPFLTQV
PPNITTTTTTTASSFLNLKSNKDTIPNNNTMLLQGGCFVGFNSTEPKSH
HVVPLGKLKGIFMSIFRFKVVWTHWVGTVNGQELQHETQMLILDKNDSL
GRPYVLLLPILNTFRTSLQPGLNDHIGMSVESGSTHVTGSSFKACLYIH
LSNDPYSILKEAVKVIQTQLGTFKTLLEKTAPSIIDKFGWCTWDAFYLV
HPKGVWEGVKSLTDGGCPPGFVIIDDGWQSI CHDDDDDDSGMNRTSAGE
QMPCLVKYEENSKFREYENPENGKKGLGGFVRDLKEEFGSVESVYVWH
ALCGYWGGVRPGVHGMFKARVVVPKVSQGLKMTMEDLAVDKIVENGVLV
PPDFAHEMFGLHSHLESAGIDGVKVDVIHLLELLSEYGGRVELARAYY
KALTSSVKKHFKGNGVIASMEHCNDFLLGTEAISLGRVGDDFWCSDPSG
DPNGTYWLQGGCHMVHCAYNSLWMGNFIQPDWDMFQSTHPCAEFHAASRAI
SGGPIYVSDCVGNHNFKLLKSLVLPDGSILRCQHYALPTRDCLFEDPLHN
GKTMKIWNLNKYTGVLGLFNCQGGWCPEARRNKSVSEFSRAVTCYASP
EDIEWCNGKTPMSTKGVDFFAVYFFKEKKRLMKCSDRCLKVSLEPFSFEL
MTVSPVKVFSKRFIQFAPIGLVNMLNSGGAIQSLEFDDNASLVKIGVRGC
GEMSVFASEKPVCKIDGVKVKFLYEDKMARVQILWPSSSTLSLVQFLF
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Sc-03:

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MAPSFSKENSKTCDDEVANHDDCNTCPIISLEESNFMVNGHVILSQVPSNI
TAISKMGFDGLFVGFDAPFKARHVSVGQLKGIPFMSIFRFKVVWTHW
TGSNGRDLEHETQILILDKSDEGLGRPYIVILPLIEGPFRASLQPGSVDD
YVDICVESGSTKVVGDSFRVLYIRAGPDPFKLIKDTMKEVQAHLGTFKL
LDDKTPPGIVDKFGWCTWDAFYLVEXYGVWEGVKGLVENGVPGLVLID
DGWQSI CHDDDPITDQEGINRTSAGEQMPCLIKYEENFKFRDYKSPNIM
GHEDHPNMGMRAFVRDLKEEFKTVHHVYVWHAFTGYWGGVRPNVPLXEA
QVVTPLKSPGLEMTMEDLAVDKIVNNGIGLVQPDKAQELYEGLHSHLENC
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GIDGVKVDVIHLLEMAEDYGGRVELAKTYKAITESVRKHFKGNGV IAS
 MEQCNDFMLLG TETICLGRVGDDFWPTDP SG DINGTYWLQGCHMVHCAYN
 SLWMGNFIHPDWD MFQSTHPCA EFHAASRAISGGPIYVSDVVGKHNIPLL
 KRLVLADG SILRCEYHALPTKDCLFVDPLHDGKTMLKIWNLNKYNGVLGV
 FNCQGGGWSRESRKNLCFSEYSKPI SCKTSPKDV EWENGHKPFP IKGVEC
 FAMYFTKEKKL ILSQLSDTIEI SLDPFDYEL I VVSPMTILPWESIAFAP I
 GLVNMLNAGGAVKSLDI SEDNEDKMVQVG IKGAGEMMVYSSEKPKACRVN
 GEDMEFEYEESMIKVQVTWNHNSGGFTTVEYLF

Sc-04 (truncated):

MAPSISKTVELNSFGLVNGNLPLSITLEGSNFLANGHPFLTEVPENI IVT
 PSPIDAKSSKNNEDDDVGCFVG FHADEPRSRHVASLGKLRGIKFMSIFR
 FKVWWTTHWVGSNGHELEHETQMMLLDKNDQLGRPFVLILPILQASFRAS
 LQPGLLDDYVDVCMESGSTRVCGSSFGSCLYVHVGHDPYQLLREATKVVRM
 HLGTFKLLEEKTA PVIIDKFGWCTWDAFYLVKHPSGVWEGVKGLVEGGCP
 PGMVLIDDGWQAI CHDEDPITDQEGMKRTSAGEQMP CRLVKLEENYKFRQ
 YCSGKDSEKGMGA FVRDLKEQFRSVEQVYVWHALCGYWGGVRPKVPGMPQ
 AKVVT PKLSNGLKLTMKDLAVDKI VSNVGLVPPHLAHLLEYGLHSRLES
 AGIDGVKVDVIHLLEMLSEEYGGRVELAKAYYKALTASVKKHFKGNGVIA
 SMEHCNDFFLLGTEAIALGRVGDDFWCTDP SGDPNGTYWLQGCHMVHCAY
 NSLWMGNFIQPDWDMFQSTHPCA EFHAPLGP SLVDQFTLVIVLESTTSSC
 SRASLCLMGRFCVVNTMHSPHETVCLKTPCMMGRQCSKFGISTNIQVFWV
 YLIAKEVGGVP

Sc-05:

MAPP SVIKSDAAVNGIDL SGKPLFRLEGSDLLANGHVVLTDVPVNVTVTA
 SPYLADKDGEPVDASAGSFIGFNLDGEPRSRHVASIGKLRDIRFMSIFRF
 KVWWTTHWVGSKGSDIENETQII ILENSGSGRPYVLLLPLLEGSFRSSFQ
 PGEDDDVAVCVESGSTQVTGSEFRQVYVHAGDDPFLVKDAMKVVRVHM
 NTFKLLEEKXPPGI VDKFGWCTWDAFYLT VNP DG VHKGVKCLVDGGCPPG
 LVLIDDGWQSIGHDSGIDVEGMSCTVAGEQMP CRLKFQENFKFRDYVS
 PKDKNEVGMAFVRDLKEEFSTVDYIYVWHALCGYWGGLRPGAPTLPPST
 IVRPELSPGLKLTMQDLAVDKI VDTGIGFVSPD MANEFYEGLHSHLQNVG
 IDGVKVDVIHILEMLCEKYGGRVDLAKAYFKALTSSVNKHFDGNGV IASM
 EHCNDFMFLGTEAIALGRVGDDFWCTDP SG DINGTYWLQGCHMVHCAYNS
 LWMGNFIQPDWDMFQSTHPCA EFHAASRAISGGPIYISDCVGQHDFDLLK
 RLVL PDGSILRCEHYALPTRDRLFEDPLHDGKTMLKIWNLNKYTG IIGAF
 NCQGGGWCRETRRNQCFSQCVNTLTATTNPKDVEWNSGNNPISVENVEEF
 ALFLSQSKKLVLSGPNDDLEITLEPFFKELITVSPVVTIEGSSVQFAPIG
 LVNMLNTSGAIRSLVYHEESVEIGVRGAGEFRVYASRK PASCKIDGEVVE
 FGYEESMVMVQVPWSAPEG LSSIKYEF

PsRFS:

MAPPSITKTATQQDVISTVDIGNSPLLSISLDQSRNFLVNGHPFLTQVPP
NITTTTTSTPSPFLDFKSNKDTIANNNNTLQQQCGFVGFNTEAKSHHV
PLGKLKGIKFTSIFRFKVVWTTTHWVGTHGHEHQHETQILILDKNISLGRP
YVLLLPILENSFRTSLQPLNDYVDMVESGTHVTGSTFKACLYLHLSN
DPYRLVKEAVKVIQTKLGTFTLEEKTPPSIEKFGWCTWDAFYLVKHPK
GVWEGVKALTDGGCPPGFVIIDDGWQSI SHDDDDPVTERDGMNRTSAGEQ
MPCRLIKYEENYKFREYENGDNNGKKGLVGFVRDLKEEFRSVESVYVWHA
LCGYWGGVRPKVCGMPEAKVVVPKLSPGVKMTMEDLAVDKIVENGVLVP
PNLAQEMFDGIHSHLESAGIDGVKVDVIHLLLELSEEYGGRVELAKAYYK
ALTSSVNKHFKNGVIAASMEHCNDFLLGTEAISLGRVGDDFWCCDPSPGD
PNGTYWLQGGCHMVHCAYNSLWMGNFIHPDWDMFQSTHPCAFAASRAIS
GGPVYVSDCVGNHNFKLLKSFVLPDGSILRCQHYALPTRDCLFEDPLHNG
KTMKLIWNLNKYAGVLGLFNCQGGGWCPEPTRRNSASEFSHAVTCYASPE
DIEWCNGKTPMDIKGVDVFAVYFFKEKKLSLMKCSDRLEVSLEPFSFELM
TVSPLKVFSKRLIQFAPIGLVNMLNSGGAVQSLEFDDASLVKIGVRGCG
ELSVFASEKPVCKIDGVSVEFDYEDKMVRVQILWPGSSTLSLVEFLF

Aj-05:

MAPSFKNGGSNVVSFDGLNDMSSPFAIDGSDFTVNGHSFLSDVPENIVAS
PSPYTSIDKSPVSVGCFVGFDASEPDSRHVVSIGKLDIRFMSIFRFKVV
WTTTHWVGRNGGDLESETQIVILEKSDSGRPYVFLLPIVEGPFRTSIQPGD
DDFVDVCVESGSSKVVDASFRSMLYLHAGDDPFALVKEAMKIVRTHLGTF
RLLEEKTPPGIVDKFGWCTWDAFYLTVHPQGVIEGVRHLVDGGCPPGLVL
IDDGWQSIGHSDPI TKEGMNQT VAGEQMPCRLKQFQENYKFRDYVNPKA
TGPRAGQKGMKAFIDELKGEFKTVEHVYVWHALCGYWGGLRPQVPGLPEA
RVIQPVLSPLQMTMEDLAVDKIVLHKVGLVPPEKAEEMYEGLHAHLEKV
GIDGVKIDVIHLLLEMLCEDYGGRVDLAKAYYKAMTKSINKHFKGNGVIAS
MEHCNDFMFLGTEAISLGRVGDDFWCTDPSGDPNGTFWLQGGCHMVHCAND
SLWMGNFIHPDWDMFQSTHPCAFAASRAISGGPIYVSDSVGKHNFDDL
KKLVLPDGSILRSEYYALPTRDCLFEDPLHNGETMLKIWNLNKFTGVIGA
FNCQGGGWCPEPTRRNCFSQYSKRVTSTNPKDIEWHSGENPISIEGVKT
FALYLYQAKKILSKPSQDLDIALDPFELITVSPVTKLIQTSLHFAPI
GLVNMLNTSGAIQSVDYDDLSSVEIGVKGCGEMRVFASKKPRACRIDGE
DVGFKYDQDMVVVQVPWPIDSSSGGISVIEYLF

HvSIP:

MTVTPQITVGDGRLAVRGRTVLSGVPDNVTAHAAGAGLVDGAFVGATAA
EAKSHHVFTFGTLRDCRFMCLFRFKLWMTQRMGTSGRDVPLETQFILIE

VPAAAGNDDGDSSDGDSEPVYLVMLPLLEGQFRTVLQGNDQDELQICIES
 GDKAVETEQQGMNNVYVHAGTNPFDITQAVKAVEKHTQTFHHREKKTGPS
 FVDWFGWCTWDAFYTDVTADGVKQGLRSLAEGGAPPRFLIIDGWQQIGS
 ENKDDPGVAVQEGAQFASRLTGIRENTKFQSEHNQEETPGLKRLVDETKK
 EHGVKSVYVWHAMAGYWGGVKPSAAGMEHYEPALAYPVQSPGVTGNQPD
 VMDSL SVLGLGLVHPRRVHRFYDELHAYLAACGVDGVKVDVQNI VETLGA
 GHGGRVALTRAYHRALEASVARNFDPNGCISCMCHNTDMLYSAKQTAVVR
 ASDDFYPRDPASHTVHISSVAYNTLFLGEFMQPDWDMFHSHPAAEYHGA
 ARAIGGCPYVSDKPGNHNFDLLRKLVLDPGSLVLAQLPGRPTRDCLFSD
 PARDGASLLKIWNMNCAGVGVFNCQGAGWCRVAKKTRIHDEAPGTLTG
 SVRAEDVEAIAQAAGTGDWGGAEVVAHRAVELVRLPRGATLPVTLKRLE
 YELFHVCPVRAVAPGVSFAPIGLLHMFNAGGAVEECTVETGEDGNAVVG
 RVRGCGRFGAYCSRRPAKCSVDSADVEFTYDSDTGLVTADVPVPEKEMYR
 CALEIRV

AmSTS:

MAPPYDPIPIPIPM SAILNFLSSTVKDNSFELLDGTL SVKNVPILTDIPS
 NVSFSSFSI VQSSEAPVPLFQRAQSLSSSGGFLGFSQNEPSSRLMNSLG
 KFTDRDFVSI FRFKTWWSTQWVGTTGSDIQMETQWIMLDVPEIKSYAVVV
 PIVEGKFRSALFPKGKDHILIGAESGSTKVKTSNFDIAIYVHVSENPYTL
 MRDAYTAVRVHLNTFKLIEEKSAPPLVNKFGWWTWDAFYLTVEPAGIYHG
 VQEFADGGLTPRFLIIDGWQSI NNDDNDPNEDAKNLVLGGTQMTARLHR
 LDECEKFRKYKGGMSGPNRPPFDPKPKLLISKAIEIEVAEKARDKAAQ
 SGVTDLARYEAEIEKLTKELDQMFGGGGEETSSGKSCSSCCKSDNFGMK
 AFTKDLRTNFKGLDDIYVWHALAGAWGGVRPGATHLNAKIVPTNLSPGLD
 GTMTDLAVVKIIEGSTGLVDPDQAEDFYDSMHSYLSVGI TGKVDVIHT
 LEYISEDYGGVELAKAYYKGLSKSLAKNFNGTGLISSMQQCNDFFLLGT
 EQISMGRVGDDFWFQDPNGDPMGVYWLQGVHMIHCAYNSMWMGQFIQPDW
 DMFQSDHPGGYFHAGSRAICGGPVYVSDSLGGHNFDLLKKLVFNDGTIPK
 CIHFALPTRDCLFKNPLFDSKITLKIWNFNKYGGVIGAFNCQGAGWDPKE
 QRIKGYSCYKPLSGSVHVSIEFDQKKEASEMGEAEYAVYLSEAELLS
 LATRSDPIKITIQSSTFEIFSFPVKKLGEGVKFAPIGLTNLFNAGGTI
 QGLVYNEGIAKIEVKGDGKFLAYSSVVPKKAYVNGAEKVFAWSGNGKLEL
 DITWYEECGGISNVTFVY

PsSTS-1:

MAPPLNSTTSNLIKTESIFDLSEKFKVKGFPLFHDVPENVSFERSFSSIC
 KPSESNAPPSLLQKVLAYSHKGGFFGFSHETPSDRLMNSIGSFNGKDFLS
 IFRFKTWWSTQWIGKSGSDLQMETQWILIEVPETKSYVVIPIIEKCFRS
 ALFPGFNDHVKIIAESGSTKVKESTFNSIAYVHFSNPYDLMKEAYSAIR
 VHLNSFRLLLEKTI PNLDKFGWCTWDAFYLT VNPIGIFHGLDDFSKGGV

EPRFVIIDDGWQSI SFDGYDPNEDAKNLVLGGEQMSGRLHRFDECYKFRK
YESGLLLGPNSPPYDPNNFTDLILKGI EHEKL RKKREEA I SSKSSDLAE I
ESKIKKVVEIDDLFGGEQFSSGEKSEMKSEYGLKAFTKDLRTKFKGLDD
VYVWHALCGAWGGVRPETTHLDTKIVPCKLSPGLDGTMEDLAVVEISKAS
LGLVHPSQANELYDSMHSYLAESGITGVKVDVIHSLEYVCDEYGGRVDLA
KVVYEGLTKSIVKNFNNGNMIASMQHCNDFFFLGTKQISMGRVGDDFWFQ
DPNGDPMGSFWLQGVHMIHCSYNSLWMGQMIQPDWDMFQSDHVC AKFHAG
SRAICGGPIYVSDNVGSHDFDLIKKLVFPDGTIPKCIYFPLPTRDCLFKN
PLFDHTTVLKIWNFNKYGGVIGAFNCQGAGWDPIMQKFRGFPECYKPIPG
TVHVTEVEWDQKEETSHLGKAEYVVYLNQAEELSLMTLKSEPIQFTIQP
STFELYSFVPVTKLCGGIKFAPIGLTNMFNSGGTVIDLEYVGNGAKIKVK
GGGSFLAYSSSESPKKFQLNGCEVDFEWLGDGKLCVNVPWIEEACGVSDME
IFF

PsSTS-2:

MAPPLNSTTSNLIKTESIFDLSEKFKVKGFPLFHDVPENVSF RSFSSIC
KPSESNA PPSLLQKVLAYSHKGGFFGFSHETPSDRLMNSLGSFNGKDFLS
IFRFTWWSTQWIGKSGSDLQMETQWILIEVPETKSYVVIPIIEKCFRS
ALFPGFNDHVKIIAESGSTKVKESTFNSIAYVHFSENOPYDLMKEAYIAIR
VHLNSFRLL E EKTIPNLVDKFGWCTWDAFYLT VNP IGI FHGLDDFSKGGV
EPRFVIIDDGWQSI SFDGCDPNEDAKNLVLGGEQMSGRLHRFDECYKFRK
YESGLLLGPNSPPYDPKKFTDLILKGI EHEKL RKKREEA I SSKSSDLAE I
ESKIKKVVEIDDLFGGEQFSSVEKSEMKSEYGLKAFTKDLRTKFKGLDD
VYVWHALCGAWGGVRPETTHLDTKFVPCKLSPGLDGTMEDLAVVEISKAS
LGLVHPSQANELYDSMHSYLAESGITGVKVDVIHSLEYVCDEYGGRVDLA
KVVYEGLTKSIVKNFNNGNMIASMQQCNDFFFLGTKQISMGRVGDDFWFQ
DPNGDPMGSFWLQGVHMIHCSYNSLWMGQMIQPDWDMFKSDHVC AKFHAG
SRAICGGPIYVSDNVGSHDFDLIKKLVFPDGTIPKCIYFPLPTRDCLFKN
PLFDHTTLLKIWNFNKYGGVIGAFNCQGAGWDPIMQKFRGFPECYKPIPG
TVHVTQVEWDQKEETSHFGKAEYVVYLNQAEELCLMTLKSEPIQFTIQP
STFELYSFVPVTKLCGGIKFAPIGLTNMFNSGGTVIDLEYVGNGAKIKVK
GGGSFLAYSSSESPKKFQLNGCEVDFEWLGDGKLCVNVPWIEEACGVS

SaSTS:

MAPPNDP I SSI FSPLISVKKDNAFELVGGKLSVKNVPLLSEIPSNVTFKS
FSSICQSSGAPAPLYNRAQSLSNCGGFLGFSQKESADSVTNSLGKFTNRE
FVSIFRFTWWSTQWVGTSGSDIQMETQWIMLNLPEIKSYAVVPIIVEGK
FRSALFPGKDGHVLI SAESGSTCVKTTSFTSIAYVHVSDNPYTLMKDGYT
AVRVHLDTFKLI E EKSAPPLVNKFGWCTWDAFYLTVEPAGI WNGVKEFS
GGFSRFLI IDDGWQSI NIDGQDPNEDAKNLVLGGTQMTARLHRFDECEK
FRKYKGGSMMPKVPYFDPKKPKLLISKAIEIEGVEKARDKAIQSGITDL

SQYEIKLKKLNKELDEMFGGGGNDEKGSSKGCSDCSCKSQNSGMKAFTND
 LRTNFKGLDDIYVWHALAGAWGGVKPGATHLNAKIEPCKLSPGLDGTMTD
 LAVVKILEGSI GLVHPDQAEDFYDSMHSYLSKVGITGVKVDVIHTLEYVS
 ENYGG RVELGKAYYKGLSKSLKKNFNGSGLISSMQQCNDFFLLGTEQISM
 GRVGDDFWFQDPNGDPMGVFWLQGVHMIHCAYNSMWMGQIIHPDWDMFQS
 DHCSAKFHAGSRAICGGPVYVSDSLGGHDFDLLKKLVFNDGTIPKCIHFA
 LPTRDCLFKNPLFDSKITLKIWNFNKYGGVVGAFNCQGAGWDPKEQRIKG
 YSECYKPLSGSVHVS DIEWDQKVEATKMGEAEYAVYLTESEKLLLTTP
 SDPIPF TLKSTTFEIFS FVPIKKLGQGVKFAPIGLTNLFNSGGTIQGVVY
 DEGVAKIEVKGDGKFLAYSSSVPKRSYLNGEVEYKWSGNGKVEVDVPWY
 EECGGISNITFVF

VaSTS:

MAPPNDPVNATLGLEPSEKVFDSLSDGKLT VKGVVLLSHVPENVTFS SFSS
 I CVPRDAPSSILQRVTAASHKGGFLGFSHVSPSDRLINSLGSFRGRNFLS
 IFRFKTWWSTQWVGNSGSDLQMETQWILIEVPETESYVVIPIIEKSFRS
 ALHPGSDDHVKICAESGSTQVRASSFGAIAYVHVAETPYNL MREAYSALR
 VHLD SFRLLEKTVPRIVDKFGWCTWDAFYLTVPVGVVHGLKDFSEGGV
 APRFVVIDDGWQSVNFDDDPNEDAKNLVLGGEQMTARLHRFEEDKFRK
 YQKGLLLGPNAPSFNPETIKELISKGIEAEHLGKQAAAI SAGGSDLAIEIE
 LMIVKVREEIDDLFGGKGKESNESGGCCCKAAECGGMKDFTTDLRTEFKG
 LDDVYVWHALCGGWGGVRPGTTHLDSKIIPCKLSPGLVGTMKDLAVDKIV
 EGSIGLVHPHQANDLYDSMHSYLAQTGVTGVKIDVHSLEYVCEEYGGRV
 EIAKAYYDGLTNSIIKNFNGSGIIASMQQCNDFFFLGTKQIPFGRVGDDF
 WFQDPNGDPMGVFWLQGVHMIHCSYNSLWMGQIIQPDWDMFQSDHECAKF
 HAGSRAICGGPVYVSDSVGSHDFDLIKKLVFPDGTVPKCIYFPLPTRDCL
 FRNPLFDQKTVLKIWNFNKYGGVIGAFNCQGAGWDPKGKKFKGFPECYKA
 ISCTVHVTEVEWDQKKEAEHMGKAEYVYVYLNQAEVLHLMTPVSEPLQLT
 IQPSTFELYNFVPVEKLGSSNIKFAPIGLTNMFNSGGTIQELEYIEKDVK
 VKVKGGGRFLAYSTQSPKKFQLNGSDAAFQWLPDGKLTNLAWIEENDGV
 SDLAIFF

The calculated overall sequence homologies (%) are shown in Table 2 attached hereto. The homologies between RFSs and SIP are less than 40%. The homologies between RFSs and STSs are not higher than 45%. On the other hand, the homologies among RFSs are all 50% or higher. Thus, the homologies among RFSs are higher than those homologies between RFSs and SIP and between RFSs and STSs.

A molecular phylogenetic tree of the RFSs, SIP and STSs shown in Table 1 is

drawn in Figure 1 attached hereto. The molecular phylogenetic tree is drawn by the UPGMA method using the gene analysis software GENETYX-SV/RC for Windows version 6.1.0 (GENETYX Corporation; <http://www.sdc.co.jp/genetyx/>) with default parameters. In the molecular phylogenetic tree, RFSs, SIP and STSs form different groups respectively.

In summary, Table 2 and Figure 1 show that RFSs, SIP and STSs can be distinguished from one another based upon a comparison of their amino acid sequences.

2) Attached Table 3 shows the identities obtained using the BLAST program for the amino acid sequences of RFSs, SIP and STSs shown in Table 1. Among Sc-02, Sc-03, Sc-04 and Sc-05, the identities were obtained by searching the "patent database" provided by NCBI (National Center for Biotechnology Information) with default parameters, using the amino acid sequence of each protein as the "query", and using "Protein query vs. translated database (tblastn)" of the NCBI BLAST program. Also, other identities were obtained by searching the "non-redundant database" provided by NCBI with default parameters, using the amino acid sequence of each protein as the "query", and using "Protein-protein BLAST (blastp)" of the NCBI BLAST program. The above-identified amino acid sequences of the RFSs, SIP and STSs are used as the "query" except that the amino acid sequence of Sc-04 used as the "query" is as follows:

Sc-04 (full-length):

```
MAPSISKTVELNSFGLVNGNLPLSITLEGSNFLANGHPFLTEVPENIIVT
PSPIDAKSSKNNEDDDVVGCFVGFHADEPRSRHVASLGKLRGIKFMSIFR
FKVWWTTHWVGSNGHELEHETQMMLLDKNDQLGRPFVLILPILQASFRAS
LQPGLLDDYVDVCMESGSTRVCGSSFGSCLYVHVGHDPYQLLREATKVVRM
HLGTFKLLEKTAPVIDKFGWCTWDAFYLVHPSGVWEGVKGLVEGGCP
PGMVLIDDGWQAI CHDEDPITDQEGMKRTSAGEQMP CRLVKLEENYKFRQ
YCSGKDSEKGMGA FVRDLKEQFRSVEQVYVWHALCGYWGGVRPKVPGMPQ
AKVVT PKLSNGLKLTMKDLAVDKIVSNGVGLVPPHLAHLLEYGLHSRLES
AGIDGVKVDVIHLLEMLSEYGRVELAKAYYKALTASVKKHFKGNGVIA
SMEHCNDFLLGTEAIALGRVGDDFWCTDP SGDPNGTYWLQGCHMVHCAY
NSLWMGNFIQPDWDMFQSTHPCA EFHAASRAISGGPVYVSDCVGKHNFKL
LKSLALPDGTILRCQHYALPTRDCLFEDPLHDGKTM LKIWNLNKYTGVLG
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LFNCQGGGWCPVTRRNKSASEFSQTVTCLASPQDIEWSNGKSPICIKGMN
VFAVYLFKDHKLKMKASEKLEVSLEPFTFELLTVSPVIVLSKKLIQFAP
IGLVNMLNTGGAIQSMEDNHIDVVKIGVRGCGEMKVFASEKPVSCKLDG
VVVKFDYEDKMLRVQVPWPSASKLSMVEFLF

As shown in Table 3, the identities between RFSs and SIPs are about 40%. The identities between RFSs and STSs range from about 40% to about 50%. On the other hand the identities among RFSs are 60% or higher. The identities among STSs are also 60% or higher. That is, the identities among RFSs or the identities among STSs are higher than the identities between RFSs and SIP or the identities between RFSs and STSs. Thus, RFSs, SIP or STSs can be distinguished based on the results of analysis using BLAST program.

3) Attached Table 4 shows the identities obtained using another BLAST program for the amino acid sequences of RFSs, SIP and STSs shown in Table 1. All possible pair-wised amino acid sequence comparison were made by the "Blast 2 Sequences" program from NCBI (<http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2.html>). Sequence identities were calculated using default parameters, program; blastp, matrix; BLOSUM62, open gap penalty; 11, extension gap penalty; 1, gap x_dropoff; 50, expect; 10.0, and word size; 3. The amino acid sequences of the RFSs, SIP and STSs used to calculate sequence identities are identical to those used as the "query" to obtain identities shown in Table 3. Results were essentially the same with former two types of comparison.

4) In conclusion, raffinose synthases (RFSs), seed imbibition protein (SIP) and stachyose synthases (STSs) were clearly distinguished from one another based on comparison of their amino acid sequences.

7. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonments, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issued thereon.

This 6th day of September, 2005


Akitsu NAGASAWA

Table 1

Code	Protein*	Organism	Accession**	Reference	Author/Assignee
Sc-03	RFS	<i>Beta vulgaris</i>	E37133	09/301,766	Sumitomo Chemical
Sc-05	RFS	<i>Brassica juncea</i>	E36417	09/301,766	Sumitomo Chemical
Sc-02	RFS	<i>Vicia faba</i>	E24423	08/992,914	Sumitomo Chemical
Sc-04	RFS	<i>Glycine max</i>	E24424	08/992,914	Sumitomo Chemical
Aj-05	RFS	<i>Cucumis sativus</i>	AF073744	Family GH36***	Ohsumi et al.
PsRFS	RFS	<i>Pisum sativum</i>	AJ426475	Family GH36	Peterbauer et al.
HvSIP	SIP	<i>Hordeum vulgare</i>	M77475	Family GH36	Heck et al.
PsSTS-1	STS	<i>Pisum sativum</i>	AJ311087	Family GH36	Peterbauer et al.
PsSTS-2	STS	<i>Pisum sativum</i>	AJ512932	Family GH36	Peterbauer et al.
VaSTS	STS	<i>Vigna angularis</i>	Y19024	Family GH36	Peterbauer et al.
AmSTS	STS	<i>Alonsoa meridionalis</i>	AJ487030	Family GH36	Voitsekhovskaja
SsSTS	STS	<i>Stachys affinis</i>	AJ344091	Family GH36	Pesch and Schmitz

*Protein: RFS, Raffinose synthase; SIP, Seed Imbibition Protein; STS, Stachyose synthase.

**Accession: GenBank Accession Number.

***Family GH36: glycoside hydrolase family 36 (see Carbohydrate-Active Enzymes (CAZy) database: http://afmb.cnrs-mrs.fr/CAZY/GH_36.html)

Table 3

[illegible]

Fig. 1

[GENETYX : Evolutionary tree]
 Date : 2004.2.4
 Method: UPGMA

